APPLICATION FOR UNITED STATES LETTERS PATENT

for

METHOD AND APPARATUS FOR PROGRAMMABLE FLUIDIC PROCESSING

by

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BACKGROUND OF THE INVENTION

1.   <u>Field of the Invention</u>

The present invention relates generally to fluidic processing and, more particularly, to a method and apparatus for programmably manipulating and interacting one or more compartmentalized packets of material on a reaction surface.

2.   <u>Description of Related Art</u>

Chemical protocols often involve a number of processing steps including metering, mixing, transporting, division, and other manipulation of fluids. For example, fluids are often prepared in test tubes, metered out using pipettes, transported into different test tubes, and mixed with other fluids to promote one or more reactions. During such procedures, reagents, intermediates, and/or final reaction products may be monitored, measured, or sensed in analytical apparatus. Microfluidic processing generally involves such processing and monitoring using minute quantities of fluid. Microfluidic processing finds applications in vast fields of study and industry including, for instance, diagnostic medicine, environmental testing, agriculture, chemical and biological warfare detection, space medicine, molecular biology, chemistry, biochemistry, food science, clinical studies, and pharmaceutical pursuits.

A current approach to fluidic and microfluidic processing utilizes a number of microfluidic channels that are configured with microvalves, pumps, connectors, mixers, and detectors. While devices using micro-scale implementations of these traditional approaches may exhibit at least a degree of utility, vast room for improvement remains. For instance, pumps and valves used in traditional fluidic transportation are mechanical. Mechanical devices, particularly when coupled to thin microchannels, may be prone to failure or blockage. In particular, thin channels may become narrowed or partially-blocked due to buildup of channel contamination, which, in turn, may lead to mechanical failure of associated devices. Current microfluidic devices also lack flexibility, for they rely upon a fixed pathway of microchannels. With fixed pathways, devices are limited in

1　　the number and type of tasks they may perform. Also, using fixed pathways makes many

2　　types of metering, transport, and manipulation difficult. With traditional devices, it is

3　　difficult to partition one type of sample from another within a channel.

4　　　　Electrical properties of materials have been employed to perform a limited

5　　number of fluidic processing tasks. For example, dielectrophoresis has been utilized to

6　　aid in the characterization and separation of particles, including biological cells. An

7　　example of such a device is described in U. S. Patent No. 5,344,535 to Betts, incorporated

8　　herein by reference. Betts establishes dielectrophoretic collection rates and collection

9　　rate spectra for dielectrically polarizable particles in a suspension. Particle concentrations

10　　at a certain location downstream of an electrode structure are measured using a light

11　　source and a light detector, which measures the increased or decreased absorption or

12　　scattering of the light which, in turn, indicates an increase or decrease in the

13　　concentration of particles suspended in the fluid. Although useful for determining

14　　particle dielectrophoretic properties, such a system is limited in application. In particular,

15　　such a system does not allow for general fluidic processing involving various

16　　interactions, sometimes performed simultaneously, such as metering, mixing, fusing,

17　　transporting, division, and general manipulation of multiple reagents and reaction

18　　products.

19　　　　Another example of using certain electrical properties for specific types of

20　　processing is disclosed in U.S. Patent No. 5,632,957 to Heller *et al.*, incorporated herein

21　　by reference. There, controlled hybridization may be achieved using a matrix or array of

22　　electronically addressable microlocations in conjunction with a permeation layer, an

23　　attachment region and a reservoir. An activated microlocation attracts charged binding

24　　entities towards an electrode. When the binding entity contacts the attachment layer,

25　　which is situated upon the permeation layer, the functionalized specific binding entity

26　　becomes covalently attached to the attachment layer. Although useful for specific tasks

27　　such as DNA hybridization, room for improvement remains. In particular, such a system,

28　　utilizing attachment sites for certain binding entities is designed for particular

29　　applications and not for general fluidic processing of a variety of fluids. More

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1 specifically, such a system is designed for use with charged binding entities that interact

2 with attachment sites.

3   Another example of processing is disclosed in U.S. Patent No. 5,126,022 to Soane

4 *et al.*, incorporated herein by reference. There, charged molecules may be moved through

5 a medium that fills a trench in response to electric fields generated by electrodes.

6 Although useful for tasks such as separation, room for improvement remains in that such

7 devices are not well suited for performing a wide variety of fluidic processing

8 interactions on a wide variety of different materials.

9   There are other examples of using dielectrophoresis for performing specific,

10 limited fluidic processing tasks. U.S. Patent No. 5,795,457 to Pethig and Burt,

11 incorporated herein by reference, disclose a method for promoting reactions between

12 particles suspended in liquid by applying two or more electrical fields of different

13 frequencies to electrode arrays. While perhaps useful for facilitating certain interactions

14 between many particles of different types, the method is not well suited for general

15 fluidic processing. U.S. Patent No. 4,390,403 to Batchelder, incorporated herein by

16 reference, discloses a method and apparatus for manipulation of chemical species by

17 dielectrophoretic forces. Although useful for inducing certain chemical reactions, its

18 flexibility is limited, and it does not allow for general, programmable fluidic processing.

19   Any problems or shortcomings enumerated in the foregoing are not intended to be

20 exhaustive but rather are among many that tend to impair the effectiveness of previously

21 known processing techniques. Other noteworthy problems may also exist; however,

22 those presented above should be sufficient to demonstrated that apparatus and methods

23 appearing in the art have not been altogether satisfactory.

24 **SUMMARY OF THE INVENTION**

25   In one respect, the invention is an apparatus for programmably manipulating a

26 packet. As used herein, "packet" refers to compartmentalized matter and may refer to a

27 fluid packet, an encapsulated packet, and/or a solid packet. A fluid packet refers to one or

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1 more packets of liquids or gases. A fluid packet may refer to a droplet or bubble of a

2 liquid or gas. A fluid packet may refer to a droplet of water, a droplet of reagent, a

3 droplet of solvent, a droplet of solution, a droplet of sample, a particle or cell suspension,

4 a droplet of an intermediate product, a droplet of a final reaction product, or a droplet of

5 any material. An example of a fluid packet is a droplet of aqueous solution suspended in

6 oil. An encapsulated packet refers to a packet enclosed by a layer of material. An

7 encapsulated packet may refer to vesicle or other microcapsule of liquid or gas that may

8 contain a reagent, a sample, a particle, a cell, an intermediate product, a final reaction

9 product, or any material. The surface of an encapsulated packet may be coated with a

10 reagent, a sample, a particle or cell, an intermediate product, a final reaction product, or

11 any material. An example of an encapsulated packet is a lipid vesicle containing an

12 aqueous solution of reagent suspended in water. A solid packet refers to a solid material

13 that may contain, or be covered with a reagent, a sample, a particle or cell, an

14 intermediate product, a final reaction product, or any material. An example of a solid

15 packet is a latex microsphere with reagent bound to its surface suspended in an aqueous

16 solution. Methods for producing packets as defined herein are known in the art. Packets

17 may be made to vary greatly in size and shape, but in embodiments described herein,

18 packets may have a diameter between about 100 nm and about 1 cm.

19 In this respect, the invention includes a reaction surface, an inlet port, means for

20 generating a programmable manipulation force upon the packet, a position sensor, and a

21 controller. The reaction surface is configured to provide an interaction site for the packet.

22 The inlet port is coupled to the reaction surface and is configured to introduce the packet

23 onto the reaction surface. The means for generating a programmable manipulation force

24 upon the packet programmably moves the packet about the reaction surface along

25 arbitrarily chosen paths. As used herein, by "arbitrarily chosen paths" it is meant that

26 paths may be chosen to have any shape about the reaction surface. Arbitrarily chosen

27 paths are not limited to movements that are predefined. Arbitrarily chosen paths may be

28 modified in an unlimited manner about the reaction surface and may hence trace out any

29 pattern. The position sensor is coupled to the reaction surface and is configured to sense

1   a position of the packet on the reaction surface. The controller is coupled to the means

2   for generating a programmable manipulation force and to the position sensor. The

3   controller is configured to adjust the programmable manipulation force according to the

4   position.

5   In other aspects, the apparatus may also include an outlet port coupled to the

6   reaction surface. The outlet port may be configured to collect the packet from the

7   reaction surface. The means for generating a manipulation force may include a conductor

8   adapted to generate an electric field. The means for generating a manipulation force may

9   include a light source. The manipulation force may include a dielectrophoretic force, an

10   electrophoretic force, an optical force, a mechanical force, or any combination thereof.

11   The position sensor may include a conductor configured to measure an electrical

12   impedance of the packet. The position sensor may include an optical system configured

13   to monitor the position of the packet. The means for generating a programmable

14   manipulation force and the position sensor may be integral.

15   In another respect, the invention is an apparatus for microfluidic processing by

16   programmably manipulating packets. The apparatus includes a reaction surface, an inlet

17   port, an array of driving electrodes, and an array of impedance sensing electrodes. As

18   used herein, an "array" refers to any grouping or arrangement. An array may be a linear

19   arrangement of elements. It may also be a two dimensional grouping having columns and

20   rows. Columns and rows need not be uniformly spaced or orthogonal. An array may also

21   be any three dimensional arrangement. The reaction surface is configured to provide an

22   interaction site for the packets. The inlet port is coupled to the reaction surface and is

23   configured to introduce the packets onto the reaction surface. The array of driving

24   electrodes is coupled to the reaction surface and is configured to generate a

25   programmable manipulation force upon the packets to direct the microfluidic processing

26   by moving the packets along arbitrarily chosen paths. The array of impedance sensing

27   electrodes is coupled to the reaction surface and is configured to sense positions of the

28   packets during the microfluidic processing.

1   In other aspects, the apparatus may also include an outlet port coupled to the

2   reaction surface. The outlet port may be configured to collect the packets from the

3   reaction surface. The apparatus may also include a controller coupled to the array of

4   driving electrodes and to the array of impedance sensing electrodes. The controller may

5   be adapted to provide a feedback from the array of impedance sensing electrodes to the

6   array of driving electrodes. The array of driving electrodes and the array of impedance

7   sensing electrodes may be integral. The apparatus may also include an integrated circuit

8   coupled to the array of driving electrodes and to the array of impedance sensing

9   electrodes. The apparatus may also include a coating modifying a hydrophobicity of the

10   reaction surface. The apparatus may also include a maintenance port.

11   In another respect, the invention is an apparatus for processing packets in a

12   partitioning medium. As used herein, a "partitioning medium" refers to matter that may

13   be adapted to suspend and compartmentalize other matter to form packets on a reaction

14   surface. A partitioning medium may act by utilizing differences in hydrophobicity

15   between a fluid and a packet. For instance, hydrocarbon molecules may serve as a

16   partitioning medium for packets of aqueous solution because molecules of an aqueous

17   solution introduced into a suspending hydrocarbon fluid will strongly tend to stay

18   associated with one another. This phenomenon is referred to as a hydrophobic effect, and

19   it allows for compartmentalization and easy transport of packets upon or over a surface.

20   A partitioning medium may also be a dielectric carrier liquid which is immiscible with

21   sample solutions. Other suitable partitioning mediums include, but are not limited to, air,

22   aqueous solutions, organic solvents, oils, and hydrocarbons. The apparatus includes a

23   chamber, a programmable dielectrophoretic array, and an impedance sensing array. As

24   used herein, a "programmable dielectrophoretic array" (PDA) refers to an electrode array

25   whose individual elements can be addressed with different electrical signals. The

26   addressing of electrode elements with electrical signals may initiate different field

27   distributions and generate dielectrophoretic manipulation forces that trap, repel, transport,

28   or perform other manipulations upon packets on and above the electrode plane. By

29   programmably addressing electrode elements within the array with electrical signals,

1 electric field distributions and manipulation forces acting upon packets may be

2 programmable so that packets may be manipulated along arbitrarily chosen or

3 predetermined paths. The chamber is configured to contain the packets and the

4 partitioning medium. The programmable dielectrophoretic array is coupled to the

5 chamber and is configured to generate a programmable dielectrophoretic force to direct

6 processing of the packets. The impedance sensing array of electrodes is integral with the

7 programmable dielectrophoretic array. The impedance sensing array of electrodes is

8 configured to sense a position of the packets within the chamber.

9 In other aspects, the apparatus may also include an integrated circuit coupled to

10 the programmable dielectrophoretic array and to the impedance sensing array of

11 electrodes. The apparatus may also include a controller coupled to the programmable

12 dielectrophoretic array and to the impedance sensing array of electrodes. The controller

13 may be adapted to provide a feedback from the impedance sensing array of electrodes to

14 the programmable dielectrophoretic array. The electrodes may be between about 1

15 micron and about 200 microns and may be spaced between about 1 micron and about 200

16 microns.

17 In another respect, the invention is a method for manipulating a packet in which

18 the following are provided: a reaction surface, an inlet port coupled to the reaction

19 surface, means for generating a programmable manipulation force upon the packet, a

20 position sensor coupled to the reaction surface, and a controller coupled to the means for

21 generating a programmable manipulation force and to the position sensor. A material is

22 introduced onto the reaction surface with the inlet port. The material is

23 compartmentalized to form the packet. A position of the packet is sensed with the

24 position sensor. A programmable manipulation force is applied on the packet at the

25 position with the means for generating a programmable manipulation force. The

26 programmable manipulation force is adjustable according to the position by the

27 controller. The packet is programmably moved according to the programmable

28 manipulation force along arbitrarily chosen paths.

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1   In other aspects, the packet may include a fluid packet, an encapsulated packet, or

2 a solid packet. The compartmentalizing may include suspending the material in a

3 partitioning medium. The material may be immiscible in the partitioning medium. The

4 reaction surface may include a coating, and the hydrophobicity of the coating may be

5 greater than a hydrophobicity of the partitioning medium. The application of the

6 programmable manipulation force may include applying a driving signal to one or more

7 driving electrodes arranged in an array to generate the programmable manipulation force.

8 The programmable manipulation force may include a dielectrophoretic force, an

9 electrophoretic force, an optical force, a mechanical force, or any combination thereof.

10 The sensing of a position may include applying a sensing signal to one or more

11 impedance sensing electrodes arranged in an array to detect an impedance associated with

12 the packet.

13   In another respect, the invention is a method of fluidic processing in which the

14 following are provided: a reaction surface, an inlet port coupled to the reaction surface, an

15 array of driving electrodes coupled to the reaction surface, and an array of impedance

16 sensing electrodes coupled to the reaction surface. One or more materials are introduced

17 onto the reaction surface with the inlet port. The one or more materials are

18 compartmentalized to form a plurality of packets. A sensing signal is applied to one or

19 more of the impedance sensing electrodes to determine a position of one or more of the

20 plurality of packets. A driving signal is applied to one or more of the driving electrodes

21 to generate a programmable manipulation force on one or more of the plurality of packets

22 at the position. One or more of the plurality of packets are interacted according to the

23 programmable manipulation force.

24   In other aspects, at least one of the plurality of packets may include a fluid packet,

25 an encapsulated packet, or a solid packet. The sensing signal and the driving signal may

26 be a single processing signal. The processing signal may include a first frequency

27 component corresponding to the sensing signal and a second frequency component

28 corresponding to the driving signal. A packet distribution map may be formed according

29 to the positions of the plurality of packets. A position of one or more obstructions on the

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1   reaction surface may be determined. The interacting of one or more packets may include

2   moving, fusing, merging, mixing, reacting, metering, dividing, splitting, sensing,

3   collecting, or any combination thereof.

4   In another respect, the invention is a method for manipulating one or more packets

5   on a reaction surface in which the following are provided: a programmable

6   dielectrophoretic array coupled to the reaction surface and an impedance sensing array of

7   electrodes integral with the programmable dielectrophoretic array. A material is

8   introduced onto the reaction surface. The material is compartmentalized to form the one

9   or more packets. A path is specified upon the reaction surface. A programmable

10   manipulation force is applied with the programmable dielectrophoretic array on the one

11   or more packets to move the one or more packets along the path. A position of the one or

12   more packets is sensed with the impedance sensing array of electrodes. Whether the

13   position corresponds to the path is monitored. The one or more packets are interacted.

14   In other aspects, at lease one of the one or more packets may include a fluid

15   packet, an encapsulated packet, or a solid packet. The method may also include sensing a

16   position of an obstruction; determining a modified path, the modified path avoiding the

17   obstruction; and applying a programmable manipulation force on the one or more packets

18   to move the one or more packets along the modified path. The specification of a path

19   may include specifying an initial position and a final position. The introduction of the

20   material may include extracting the material with a dielectrophoretic extraction force

21   from an injector onto the reaction surface. The interacting of one or more packets may

22   include moving, fusing, merging, mixing, reacting, metering, dividing, splitting, sensing,

23   collecting, or any combination thereof.

24   Other features and advantages of the present invention will become apparent with

25   reference to the following description of typical embodiments in connection with the

26   accompanying drawings wherein like reference numerals have been applied to like

27   elements, in which:

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BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a simplified schematic diagram that illustrates a microfluidic device according to one embodiment of the presently disclosed method and apparatus.

FIG. 2 is a simplified illustration of dielectrophoretic force phenomenon.

FIG. 3 illustrates a position sensing system according to one embodiment of the presently disclosed method and apparatus.

FIG. 4 is a three dimensional view of a microfluidic device according to one embodiment of the presently disclosed method and apparatus.

FIG. 5 is a side cross sectional view of a microfluidic device according to one embodiment of the presently disclosed method and apparatus.

FIG. 6 is a simplified block representation of a microfluidic system according to one embodiment of the presently disclosed method and apparatus.

FIG. 7 is a simplified block representation of a signal application arrangement according to one embodiment of the presently disclosed method and apparatus.

FIG. 8 is a cross sectional view of microfluidic device according to one embodiment of the presently disclosed method and apparatus.

FIG. 9 is a top view of a microfluidic device according to one embodiment of the presently disclosed method and apparatus.

FIG. 9B is another top view of a microfluidic device according to one embodiment of the presently disclosed method and apparatus.

FIG. 10 is a simplified block representation of a microfluidic system according to one embodiment of the presently disclosed method and apparatus.

1   **FIG. 11** is a top view of a microfluidic device showing a microfluidic process

2 according to one embodiment of the presently disclosed method and apparatus.

3   **FIG. 12** illustrates certain packet interactions according to one embodiment of the

4 presently disclosed method and apparatus.

5   **FIG. 13** is a flow chart showing a microfluidic process according to one

6 embodiment of the presently disclosed method and apparatus.

7

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

9   The disclosed method and apparatus provide many advantages. For instance, they

10 permit the fluidic processing of minute quantities of samples and reagents. The apparatus

11 need not use conventional hardware components such as valves, mixers, pump. The

12 apparatus may be readily miniaturized and its processes may be automated or

13 programmed. The apparatus may be used for many different types of microfluidic

14 processing and protocols, and it may be operated in parallel mode whereby multiple

15 fluidic processing tasks and reactions are performed simultaneously within a single

16 chamber. Because it need not rely on narrow tubes or channels, blockages may be

17 minimized or eliminated. Further, if obstructions do exist, those obstructions may be

18 located and avoided with position sensing techniques.

19   Allowing for flexible microfluidic processing, the disclosed method and apparatus

20 has vast applications including, but not limited to, blood and urine assays, pathogen

21 detection, pollution monitoring, water monitoring, fertilizer analysis, the detection of

22 chemical and biological warfare agents, food pathogen detection, quality control and

23 blending, massively parallel molecular biological protocols, genetic engineering,

24 oncogene detection, and pharmaceutical development and testing.

25   In one embodiment of the disclosed method and apparatus, a fluidic device **10** as

26 shown in **FIG. 1** is employed. As illustrated, fluidic device **10** may include a reaction

surface 12, a port 15, packets 21, wall 22, position sensor 23, a force generator 25, and a controller 81.

In operation, one or more materials may be introduced onto reaction surface 12 through port 15. The one or more materials may be compartmentalized to form packets 21 within a partitioning medium (not shown). Force generator 25 generates a manipulation force on packets 21 to facilitate fluidic manipulations and interactions. In the illustrated embodiment, force generator 25 generates two forces, F_1 and F_2, that manipulate packets 21 and moves them according to the dashed lines of **FIG. 1**. Position sensor 23 senses the positions of packets 21 and is able to monitor any packet interactions. As position sensor 23 is coupled to force generator 25 by controller 81, a feedback relationship may be established. Such feedback may include determination of the position of packets 21 on reaction surface 12 that allows for the application of manipulation forces on packets 21 based on position information. The position of packets during manipulation may thus be continuously monitored and this information may be used to continuously adjust one or more manipulation forces so to achieve movement of packets 21 along a desired trajectory to a desired location on reaction surface 12.

In the illustrated embodiment of **FIG. 1**, forces F_1 or F_2 may include many different types of forces. For instance, forces F_1 and F_2 may be dielectrophoretic, electrophoretic, optical (as may arise, for example, through the use of optical tweezers), mechanical (as may arise, for example, from elastic traveling waves or from acoustic waves), or any other suitable type of force (or combination thereof). In one embodiment, forces F_1 and F_2 may be programmable. Using programmable forces, packets may be manipulated along arbitrarily chosen paths.

In the illustrated embodiment of **Fig. 1**, position sensor 23 may be operated with various mechanisms to sense positions of packets 21. For instance, an optical imaging system may be used to determine and monitor packet positions. Specifically, an optical microscope may be connected to a CCD imaging camera, which may be interfaced with

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1   an imaging card in a computer. The information from the imaging card may be processed

2   in the computer using image-analysis software. Alternatively, a CCD imaging device

3   may be incorporated in or above the reaction surface 12 to monitor the positions of

4   packets. Thus, positions of packets and their movement on reaction surface 12 may be

5   continuously monitored and recorded in the computer. A different mechanism of packet

6   position sensing uses electrical impedance measurements. The presence or absence of a

7   packet between two electrode elements may affect the electrical impedance between the

8   electrodes. Thus, measurement of electrical impedance between electrode elements may

9   allow for indirect monitoring of packet positions.

10   In order to better understand the operation and design of the currently disclosed

11   method and apparatus, which will be discussed first in relation to dielectrophoretic forces,

12   it is useful to discuss dielectrophoretic theory in some detail. Such a discussion is aided

13   by **FIG. 2**, which illustrates two packets, **21a** and **21b**, both being subjected to

14   dielectrophoretic forces.

15   Dielectrophoretic forces may arise when a packet is placed in an inhomogeneous

16   electrical field (AC or DC). In **Fig. 2** the electrical field is weaker on the left side than on

17   the right side. An electrical field induces electrical polarizations in the packet. The

18   polarization charges are depicted at the two ends of the packets **21a** and **21b** along the

19   field lines **35**. Dielectrophoretic forces result from the interaction between the induced

20   polarization (labeled as m_1 and m_2 in **FIG. 2**) and the applied inhomogeneous field. If a

21   packet is suspended in a medium having different dielectric properties, such as a

22   partitioning medium, then the packet may remain compartmentalized and may readily

23   respond to manipulation forces against viscous drag. In a field of non-uniform strength, a

24   packet may be directed towards either strong (packet **21a**) or weak (packet **21b**) electrical

25   field regions, depending on whether the packet is more (packet **21a**) or less (packet **21b**)

26   polarizable than a partitioning medium. In a field of non-uniform phase distribution (i.e.

27   a traveling electrical field), a packet may be directed towards field regions of larger or

28   smaller phase distribution, depending whether the packet has a longer or shorter dielectric

29   response time than that of a partitioning medium.

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1 *DEP theory*

2 When a packet of radius r, suspended in an immiscible medium of different

3 dielectric properties, is subjected to an electrical field of frequency f, the polarization of

4 the packet can be represented using an effective dipole moment (Wang *et al.*, "A Unified

5 Theory of Dielectrophoresis and Traveling Wave Dielectrophoresis", Journal of Physics

6 D: Applied Physics, Vol 27, pp. 1571-1574, 1994, incorporated herein by reference)

7
$$\vec{m}(f) = 4\pi\varepsilon_m \, r^3 P_{CM}(f)\vec{E}(f) \tag{1}$$

8 where $\vec{m}(f)$ and $\vec{E}(f)$ are the dipole moment and field vectors in the frequency domain,

9 $P_{CM}(f)$ is the so-called Clausius-Mossotti factor, given by

10
$$P_{CM}(f) = (\varepsilon_d^* - \varepsilon_m^*)/(\varepsilon_d^* + 2\varepsilon_m^*). \tag{2}$$

11 Here $\varepsilon_k^* = \varepsilon_k - j\sigma_k/(2\pi f)$ are the complex permittivities of the packet material

12 ($k = d$) and its suspension medium ($k = m$), and ε and σ refer to the dielectric

13 permittivity and electrical conductivity, respectively. Using the effective dipole moment

14 method, the DEP forces acting on the packet are given by

15
$$\vec{\vec{F}}(f) = 2\pi \, r^3 \varepsilon_m \left(\mathrm{Re}[P(f)]\nabla E_{(rms)}^2 + \mathrm{Im}[P(f)]\left(E_{x0}^2 \nabla\varphi_{x0} + E_{y0}^2 \nabla\varphi_{y0} + E_{z0}^2 \nabla\varphi_{z0}\right)\right) \tag{3}$$

16 where $E(rms)$ is the RMS value of the field strength, E_{i0} and φ_{i0} (i=x; y;z) are the

17 magnitude and phase, respectively, of the field components in a Cartesian coordinate

18 frame. Equation (3) shows that the DEP force contains two independent terms. The first,

19 relating to the real (in phase) part of the polarization factor $\mathrm{Re}[P(f)]$ and to non-

20 uniformities in the field *magnitude* ($\nabla E_{(rms)}^2$). Depending on the sign of $\mathrm{Re}[P(f)]$, this

21 force directs the packet either toward strong or weak field regions. The second term

22 relates to the imaginary (out of phase) part of the polarization factor ($\mathrm{Im}[P(f)]$) and to

23 field phase non-uniformities ($\nabla\varphi_{i0}$, i=x; y; z) that correspond to the field traveling

24 through space from large to small phase regions. Depending on the sign of $\mathrm{Im}[P(f)]$,

25 this directs packets toward regions where the phase values of the field components are

26 larger or smaller.

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1   Equations (1-3) indicate that the DEP phenomena have the following

2   characteristics:

3   (1)   DEP forces experienced by packets are dependent on the dielectric

4   properties of the packets (ε_d^*) and the partitioning medium (ε_m^*).

5   (2)   The strong dependence of three-dimensional DEP forces on the field

6   configuration allows for versatility in implementing dielectrophoretic manipulations.

7   *DEP Forces on Packets*

8   In one embodiment, a conventional dielectrophoresis component may be used for

9   packet manipulation. In this case, the DEP force is given by

10   $$\bar{\bar{F}}(f) = 2\pi\ r^3 \varepsilon_m\ \mathrm{Re}[P(f)]\nabla E^2_{(rms)} \tag{4}$$

11   where r is the packet radius, ε_m is the dielectric permittivity of the suspending

12   fluid. $\mathrm{Re}[P(f)]$ is the real (in phase) part of the polarization factor and $\nabla E^2_{(rms)}$ is the

13   field non-uniformity factor. For packets of water (ε = 78 and $\sigma > 10^{-4}$ S/m) suspended

14   in a hydrocarbon fluid (ε = ~ 2 and σ ~ 0), the factor $\mathrm{Re}[P(f)]$ is always positive and

15   close to unity. Therefore, water packets are always attracted towards regions of large

16   field strength. For example, if an electrode array composed of circular electrodes

17   arranged in a hexagonal fashion is provided, water packets may be dielectrophoretically

18   moved towards and trapped between, for example, an electrode pair, over a single

19   electrode, or above a plurality of electrodes to which electrical signals are applied.

20   Switching the electrical signals may result in movement of the DEP traps and may cause

21   water packets to move in a chamber. Thus, packet manipulation may be realized by

22   switching electrical signals applied to an electrode array so that DEP field traps are made

23   "mobile" within a chamber.

2   For a water packet of 100 μm suspended in a hydrocarbon fluid such as decane,

3 the DEP force may be on the order of 1000 pN if the field non-uniformity is 1.25×10^{13}

4 V^2/m^3 (equivalent to 5V RMS applied to an electrode pair of distance 50 μm with the

5 field decaying to zero at 1000 μm). If the viscosity of the hydrocarbon fluid is small

6 (0.838 mPa for Decane), the packet velocity may be of the order of 600 μm/sec,

7 indicating that fast manipulation of packets is possible with electrode arrays. In the

8 above analysis, DEP force equation (4) has been used, which was developed for non-

9 deformable particles and holds well for suspended particles (such as cells, latex particles).

10 Fluid packets may be deformed under the influence of applied electrical field, affecting

11 the accuracy of equation (4) in describing DEP forces for packets. Nevertheless,

12 equation (4) should be generally applicable with some possible correction factors for

13 different packet shapes.

14   **FIG. 3** shows one possible implementation of position sensor **23** of **FIG. 2**.

15 Shown in **FIG. 3** are five impedance sensing electrodes **19**, here illustrated as **19a**, **19b**,

16 **19c**, **19d**, and **19e**. Each sensing electrode **19** may be coupled to an impedance sensor **29**,

17 here illustrated as impedance sensors **29a**, **29b**, **29c**, and **29d**. In one embodiment,

18 impedance sensing electrodes **19** may be positioned in operative relationship with surface

19 **12** of fluidic device **10** in **FIG. 1**. For instance, sensing electrodes **19** may be placed on

20 or near surface **12**. As packets **21** are manipulated about surface **12** by the application of

21 appropriate manipulation forces, impedance sensing electrodes **19** and sensors **29** may

22 sense a position of packets **21** by making one or more impedance measurements.

23   If the dielectric medium above an electrode is displaced by a packet having

24 different dielectric and/or conductive properties, the impedance detected at the electrode

25 element will change. Thus, one may determine the position of packets **21** by noting the

26 impedance measurements associated therewith. As is shown in **FIG. 3**, the impedance

27 between impedance sensing electrodes **19a** and **19b** is "high" (see impedance sensor **29d**)

28 relative to, for instance, the impedance between impedance sensing electrodes **19b** and

1   **19c** (see impedance sensor **29c**). Thus, by pre-determining that the "high" impedance

2   value corresponds to the impedance due to the partitioning medium, it may be deduced

3   that some material of different impedance to the partitioning medium lies somewhere

4   between impedance sensing electrodes **19d** and **19e** and between **19b** and **19c** because the

5   impedance associated with those electrodes is " low" (see impedance sensor **29a**). By

6   like reasoning, one may assume that no packet lies between impedance sensing electrodes

7   **19c** and **19d**, for the impedance between those two electrodes is relatively " high" (see

8   impedance sensor **29b** and **29c**).

9   Those of skill in the art will appreciate that the "low" and "high" values discussed

10   above may be reversed, depending upon the relative impedances of a packet and of a

11   suspending medium. In other words, in some situations, a relatively "high" impedance

12   measurement may signal the presence of a packet in between a pair of electrodes while a

13   relatively "low" impedance may signal the lack of a packet. Those of skill in the art will

14   also appreciate that individual impedance measurements may exhibit a wide range of

15   values (not just "low" or "high"), and it may be possible to characterize different types

16   and sizes of materials by noting their associated impedance measurements. For instance,

17   one may be able to differentiate, by type, the two packets **21** of **FIG. 3** by noting any

18   differences in their impedance readings on impedance sensors **29a** and **29c**.

19   Impedance sensing may be based on the so-called mixture theory, which

20   associates the impedance of a heterogeneous system with the dielectric properties of

21   various system components and their volume fractions. Take a two-component,

22   heterogeneous system where component 2 having complex dielectric permittivity

23   ($\varepsilon_2^* = \varepsilon_2 - j\dfrac{\sigma_2}{2\pi f}$, f is the frequency) and a volume fraction α is suspended in

24   component 1 having complex dielectric permittivity ($\varepsilon_1^* = \varepsilon_1 - j\dfrac{\sigma_1}{2\pi f}$). The complex

25   permittivity of the total system is given by (Wang *et al.*, "Theoretical and experimental

26   investigations of the interdependence of the dielectric, dielectrophoretic and

1 electrorotational behavior of colloidal particles" in J. Phys. D: Appl. Phys. 26: 312-322,

2 1993, incorporated herein by reference)

$$\varepsilon_{sys}^* = \varepsilon_1^* \frac{\dfrac{1}{\alpha} + 2\dfrac{\varepsilon_2^* - \varepsilon_1^*}{\varepsilon_2^* + 2\varepsilon_1^*}}{\dfrac{1}{\alpha} - \dfrac{\varepsilon_2^* - \varepsilon_1^*}{\varepsilon_2^* + 2\varepsilon_1^*}}.$$

4 The total impedance of the system, which is assumed to have length L and cross-

5 sectional area A, is given by

$$\Omega = \frac{L}{\omega \varepsilon_{sys}^* A}.$$

7 The electrical impedance between two electrode elements in the presence or

8 absence of a packet may be analyzed using the above equations, with the parameters L

9 and A determined experimentally. The existence of a packet may correspond to $\alpha > 0$ and

10 the absence of a packet may correspond to $\alpha = 0$. From these equations, an impedance

11 change would occur when a packet having different dielectric property (ε_2^*) from the

12 partitioning media (ε_1^*) is introduced into the space between the two electrode elements.

13 A relatively low impedance measurement may indicate an obstruction or a packet

14 (as is illustrated in **FIG. 3**) on or near surface **12**. By determining impedance values, one

15 may map locations of obstructions or packets relative to surface **12**. In this way, one may

16 generate a packet and/or obstruction distribution map with respect to reaction surface **12**

17 of fluidic device **10**. With the benefit of this disclosure, one of skill in the art will

18 appreciate that the description associated with **FIG. 3** may be implemented in many

19 different ways. In particular, one may use any suitable type of impedance measurement

20 devices known in the art to function with one or more electrodes. Such devices may

21 include an impedance analyzer, a DC/AC conductance meter, or any circuit based upon

22 methods of operation of these or other instruments having similar function.

1   **FIG. 4** shows a three dimensional view of one embodiment of a fluidic device **10**

2   according to the present disclosure. Fluidic device **10** includes reaction surface **12**, an

3   inlet port **14**, an outlet port **16**, driving electrodes **18**, impedance sensing electrodes **19**,

4   connectors **20**, and wall **22**.

5   Reaction surface **12** provides an interaction site for packets. In one embodiment,

6   reaction surface **12** may be completely or partially covered with a partitioning medium

7   (not shown in **FIG. 4**) or other substance. In one embodiment, reaction surface **12** may

8   be coated. In particular, for manipulation of aqueous packets in a hydrophobic

9   partitioning medium, reaction surface **12** may include a hydrophobic coating, or layer,

10   having a hydrophobicity similar to or greater than the hydrophobicity of the partitioning

11   medium. Such a coating may prevent an aqueous packet from sticking, from spreading,

12   or from becoming unstable upon contact with reaction surface **12**. Additionally, a coating

13   may modify association and/or interaction forces between packets and reaction surfaces

14   to facilitate manipulation of packets by appropriate manipulation forces. Further, a

15   coating may be used to reduce contamination of reaction surfaces by reagents in packets.

16   Still further, a coating may facilitate the deliberate adhesion, wetting, or sensing of

17   packets at or on reaction surfaces. If a dielectric layer coating is applied, the layer should

18   be made sufficiently thin to allow AC electric field penetration through the dielectric

19   layer. In one embodiment, the thickness of the layer may be between about 2 nm and

20   about 1 micron. In one embodiment, a hydrophobic coating may be Teflon that may be

21   applied by means known in the art such as sputtering or spin-coating. It is to be

22   understood that any other suitable coating that modifies an interaction between packets

23   and the reaction surface may be used.

24   Reaction surface **12** may be formed from a number of suitable materials. In the

25   illustrated embodiment, reaction surface **12** is a planar surface that has an upper surface

26   including driving electrodes **18** and impedance sensing electrodes **19**. Although

27   illustrated as being coplanar with reaction surface **12**, it is to be understood that driving

28   electrodes **18** and **19** may also be elevated or depressed with respect to reaction

29   surface **12**. Likewise, reaction surface **12** need not be planar. Rather, it may have

1 concave or convex portions, or it may be deformed in some other manner. Reaction

2 surface **12** may be glass, silicon dioxide, a polymer, a ceramic, or any suitable electrically

3 insulating material. The dimensions of reaction surface **12** may vary widely depending

4 on the application but may be between about 20 microns by about 20 microns and about

5 50 centimeters by about 50 centimeters. More particularly, reaction surface **12** may be

6 between about 3 millimeters by about 3 millimeters and about 30 centimeters by about 30

7 centimeters.

8 Inlet port **14** may be adapted to inject or introduce materials onto reaction

9 surface **12** and may be any structure allowing ingress to reaction surface **12**. In the

10 illustrated embodiment, inlet port **14** consists of an opening in wall **22**. Such an opening

11 may be of any suitable size or shape. Alternatively, inlet port **14** may be a syringe needle

12 a micropipette, a tube, an inkjet injector, or any other suitable device able to inject a

13 material for introduction onto reaction surface **12**. Using a micropipette or equivalent

14 device, wall **22** may not need to include any openings. Rather, material may be

15 introduced onto reaction surface **12** from above. A micropipette or any other equivalent

16 device may be attached to a micromanipulation stage (not shown in **FIG. 4**) so that

17 material may be precisely deposited onto specific locations of reaction surface **12**. In one

18 embodiment, inlet port **14** may consist of a cylindrical tube opening onto reaction

19 surface **12**. Such a tube may have a diameter of between about 1 micrometer and about 1

20 mm and, more particularly, between about 10 and 100 microns.

21 Outlet port **16** may be adapted to collect packets of material from reaction surface

22 **12**. Outlet port **16** may be any structure allowing egress from reaction surface **12**. In the

23 illustrated embodiment, outlet port **16** consists of an opening in wall **22**. The opening

24 may be of any suitable size or shape. Alternatively, outlet port **16** may be a micropipette

25 or any other equivalent device able to collect a material from reaction surface **12**. Wall

26 **22** may not need to include any openings. Rather, packets of material may be collected

27 from reaction surface **12** from above. A syringe or any other equivalent device may be

28 attached to a micromanipulation stage (not shown in **FIG. 4**) so that packets may be

29 precisely collected from specific locations on reaction surface **12**. In one embodiment,

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1 outlet port **16** may consist of a cylindrical tube opening onto reaction surface **12**. Such a

2 tube may have a diameter of about 1 millimeter and a length of about 3 centimeters or

3 longer.

4 In one embodiment, inlet port **14** and outlet port **16** may be integral. For instance,

5 in the embodiment shown in **FIG. 1** port **15** is a cylindrical tube opening onto reaction

6 surface **12**. In alternative embodiments, one micropipette may serve as both an inlet port

7 and an outlet port. Alternatively, a single opening in wall **22** may serve both input and

8 output functions. In another embodiment, multiple inlet and outlet ports may be utilized.

9 Fluidic device **10** may include an arbitrary number of inlet and outlet ports. For

10 example, any one of the three unnumbered openings in wall **22**, illustrated in **FIG. 4**, may

11 serve as an inlet port, an outlet port, or an integral inlet-outlet port, such as port **15** of

12 **FIG. 1**. In another embodiment, multiple inlet and/or outlet ports may extend completely

13 or partially along a wall **22** so that materials may be introduced and/or collected to and/or

14 from reaction surface **12**. In such an embodiment, one may more precisely introduce or

15 collect materials.

16 In **FIG. 4**, driving electrode **18** is one of a number of other driving electrodes

17 arranged in an array upon reaction surface **12**. In this embodiment, driving electrodes **18**

18 may be associated with force generator **25** of **FIG. 1**, for the driving electrodes **18** may

19 contribute to the generation of forces, such as forces F_1 and F_2 of **FIG. 1**, to manipulate

20 packets of material on reaction surface **12** to promote, for instance, microfluidic

21 interactions.

22 Dielectrophoretic forces may be generated by an array of individual driving

23 electrodes **18** fabricated on an upper surface of a reaction surface **12**. The driving

24 electrode elements **18** may be individually addressable with AC or DC electrical signals.

25 Applying an appropriate signal to driving electrode **18** sets up an electrical field that

26 generates a dielectrophoretic force that acts upon a packet, known to be at a certain

27 location through impedance measurements as described above in relation to **FIG. 3**.

28 Switching different signals to different electrodes sets up electrical field distributions

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1   within fluidic device **10**. Such electrical field distributions may be utilized to manipulate

2   packets in a partitioning medium.

3   In particular, the movement of packets under the influence of a manipulation force

4   may be controlled by switching appropriate electrical signals to different combinations of

5   driving electrodes **18**. Specifically, the switching of electrical signals may initiate

6   different field distributions and generate manipulation forces that trap, repel, transport, or

7   perform other manipulations upon packets of material. By programmably switching

8   electrical signals to different combinations of driving electrodes **18** within an array,

9   electric field distributions and manipulation forces acting upon packets may be

10   programmable so that packets may be manipulated along arbitrarily chosen or

11   predetermined paths in a partitioning medium along reaction surface **12**. Thus, packets

12   may be manipulated in an unlimited manner. Signals may be appropriately switched to

13   cause, for instance, a packet to move a single "unit distance" -- a distance between two

14   neighboring electrodes. Further, by programmably switching electrical signals, different

15   microfluidic reactions may be performed in series or in parallel. An electrode array

16   having such an ability to utilize programmable dielectrophoretic forces by programmed

17   switching of electrical signals to different combinations of driving electrodes **18** may be

18   termed a programmable dielectrophoretic array (PDA).

19   In **FIG. 4**, impedance sensing electrode **19** is one of a number of other impedance

20   sensing electrodes arranged in an array upon reaction surface **12**. In this embodiment,

21   impedance sensing electrodes **19** may be associated with position sensor **23** of **FIG. 1** and

22   is illustrated in **FIG. 3**. Impedance sensing electrodes **19** contribute to the sensing of

23   packet positions upon reaction surface **12** so that those packets of material may be

24   monitored and manipulated according to position.

25   In the illustrated embodiment, driving electrodes **18** and impedance sensing

26   electrodes **19** are electrodes of a two dimensional electrode array coupled to a top surface

27   of reaction surface **12**. The size of the array may vary according to need, but in one

28   embodiment a 16 by 16 array is employed. Because fluidic device **10** is scaleable,

1 smaller or larger arrays may be fabricated without significant departure from the present

2 disclosure. For example, 256 by 256 arrays or larger may be made according to the

3 present disclosure. Driving electrodes 18 and impedance sensing electrodes 19 within an

4 array may be uniformly or non-uniformly spaced. The spacing may vary widely, but in

5 one embodiment, the spacing may be between about 2 microns and about 200 microns.

6 The electrodes may have different forms such as lines, squares, circles, diamonds,

7 polygons, or other suitable shapes. The dimensions of each electrode may vary, but a

8 typical electrode may be between about 0.2 microns and about 10 mm., and more

9 particularly, between about 1 micron and about 200 microns. Driving electrodes 18 and

10 impedance sensing electrodes 19 may be formed using any method known in the art. In

11 one embodiment, such electrodes may be formed using standard photolithography

12 techniques. For example, one may refer to, e.g., D. Qin et al, "Microfabrication,

13 Microstructures and Microsystems", Microsystem Technology in Chemistry and Life

14 Sciences (Ed. Manz and Becker), Springer, Berlin, 1997, pp 1- 20, which is incorporated

15 herein by reference. Also, one may refer to Madou, Fundamentals of Microfabrication,

16 CRC Press, Boca Raton, 1997, which is incorporated herein by reference. Depending

17 upon the particular application, and the nature of the packets and partitioning medium,

18 the size and spacing of electrodes 18 and 19 may be smaller than, of similar size, or larger

19 than the diameters of the packets.

20 In one embodiment, impedance sensing electrodes 19 may be integral with

21 driving electrodes 18. In such an embodiment, the resulting array may be termed an

22 integral array. With an integral array, a single conductor coupled to reaction surface 12

23 may serve both purposes -- driving packets and sensing positions of packets. Thus, a

24 programmable manipulation force may be generated upon packets upon reaction surface

25 12 and a position of those packets may be sensed with a single electrode array.

26 In the embodiment of **FIG. 4**, wall 22 is adapted to enclose one or more sides of

27 reaction surface 12. It is to be understood that wall 22 may be any suitable structure

28 capable of enclosing one or more sides and/or the top of reaction surface 12. As

29 illustrated, wall 22 encloses four sides of reaction surface 12, defining an open reaction

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surface chamber. In a most typical embodiment, the chamber may have a thickness of between about 10 microns and about 20 millimeters. In another embodiment, wall **22** may enclose the top of reaction surface **12**, forming a closed reaction chamber.

Wall **22** may be formed from any suitable material. In one embodiment, wall **22** may be made from machined plastic, aluminum, glass, plastic, ceramic, or any combination thereof. In one embodiment, wall **22** may be partially or completely transparent to certain wavelengths of radiation. Thus, radiation may be transmitted through wall **22** to initiate or maintain certain microfluidic reactions or processes for sensing. For instance, a photochemical reaction may be initiated through wall **22**.

Connectors **20** of **FIG. 4** may be adapted to provide electrical connections to driving electrodes **18** and to impedance sensing electrodes **19**. Connectors **20** may provide electrical connections to an entire array of electrodes, or to preselected ones or groups. In one embodiment, connectors **20** are coupled to a controller (not shown in **FIG. 4**) that may adjust a programmable manipulation force distribution generated by driving electrodes **18** according one or more packets position sensed with impedance sensing electrodes **19**. Thus, such a controller may effectively provide a feedback mechanism between the driving electrodes **18** and the impedance sensing electrodes **19** -- The signals applied to driving electrodes **18** may be adjusted in view of one or more results from the impedance sensing electrodes **19**.

Turning now to **FIG. 5**, there is shown a side cross section view of a fluidic device **10** according to the present disclosure. Fluidic device **10** includes a reaction chamber **41** and an array of integral impedance sensing and driving electrodes, integral array **43**. In the illustrated embodiment, a control chip **60** is coupled to integral array **43**. Positioned upon a top surface of control chip **60** may be capillary wall **62** that forms a lower surface of a capillary **64**. Capillary **64** may lead to an inlet port **14** that leads into chamber **41**. Although illustrated with only one inlet port, it is contemplated that there may be several such ports providing access to chamber **41**. Above capillary **64** is a

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1   substrate 66 that, in one embodiment, is made of glass although any suitable material

2   known in the art may be utilized instead.

3   In one embodiment, control chip 60 may be an integrated circuit configured to

4   control integrated array 43. Alternatively, control chip 60 may be a control interface

5   leading to another controlling device such as an integrated circuit, computer, or similar

6   device that may control integral array 43. Control chip 60 may utilize flip-chip

7   technology or any other suitable technique to establish electrical control over integral

8   array 43 by switching different signals to and from it.

9   FIG. 6 shows a controller 81 according to one embodiment of the presently

10   disclosed method and apparatus. Controller 81 may include a computer 80, a signal

11   generator 82, an electrode selector 84, a transducer 88, and a fluidic device 10 having a

12   driving electrode 18 and an impedance sensing electrode 19.

13   Computer 80 may be configured to control fluidic device 10 and the fluid

14   processing occurring upon reaction surface 12. Computer 80 may have a user interface

15   that allows for simple programming of signal generator 82 and transducer 88, which

16   measures impedance, to allow for programmable microfluidic processing. In particular,

17   computer 80 may programmably control the initiation/termination of one or more signals

18   from signal generator 82, the parameters of the one or more signals including frequencies,

19   voltages, and particular waveforms, and control the switching of one or more signals

20   from generator 82 to different combinations of electrodes 18 and 19.

21   Computer 80 may vary signals in many ways. For instance, one signal having a

22   first frequency component may be sent through electrode selector 84 to a driving

23   electrode 18 while another signal having a second, different frequency component may be

24   sent to, for instance, an impedance sensing electrode 19 and through electrode

25   selector 84. Any sequence of signals or combinations of signals may be sent different

26   combinations of electrodes and from the fluidic device 10. Any signal parameter may be

27   varied and any electrode selection may be controlled so that appropriate electric fields

-26-

1   may be established at particular locations upon reaction surface **12**. Alternating Current

2   or Direct Current signals may be utilized.

3   Signal generator **82** may send a driving signal to one or more driving electrodes

4   **18** while sending a sensing signal to one or more impedance sensing electrodes **19**. In

5   one embodiment, the driving signal and the sensing signal may comprise a single,

6   composite processing signal having different frequency components. Such a signal may

7   be used with an integrated array to provide (*via* a single processing signal) a frequency

8   component to generate a programmable manipulation force and a frequency component to

9   provide an impedance sensing signal. The manipulation and impedance sensing

10   components may also be combined by multiplexing or switching in time as is known in

11   the art.

12   In one embodiment, signal generator **82** provides one or more programmable

13   driving signals to one or more driving electrodes **18** through electrode selector **84** so that

14   a programmable alternating-current electric field, such as a non-uniform field, may be

15   produced at reaction surface **12**. That electric field may induce polarization of packets of

16   materials adjacent to or in the vicinity of the one or more driving electrodes **18**. A

17   programmable dielectrophoretic force distribution may, in this manner, be generated that

18   manipulates packets in a controllable, programmable manner so that varied

19   programmable fluidic interactions may take place upon reaction surface **12**.

20   In one embodiment, signal generator **82** provides a sensing signal to one or more

21   impedance sensing electrodes **19** so that an impedance measurement may be made. The

22   impedance sensing signal may be applied to one or more pairs of impedance sensing

23   electrodes **19** and a change in voltage or current may be detected and transmitted to

24   computer **80** *via* sensing electrodes **88** and wire **86**. Computer **80** may then compute the

25   impedance and hence, determine whether a packet or obstruction was present at or near

26   the pair(s) of impedance sensing electrodes **19** being probed.

27   In an embodiment utilizing a single integrated array (instead of separate

28   impedance sensing and driving electrode arrays, an integrated array utilizes electrodes

-27-

1   that function to both drive and sense packets), the integrated array may both generate a

2   programmable manipulation force and sense an impedance. In one approach, electrical

3   sensing signals for sensing electrode impedance may be applied at different frequencies

4   from driving signals for manipulation of packets. Summing signal amplifiers (not

5   shown) may be used to combine signals from sensing and driving electronics. By using a

6   frequency filter network (not shown), electrode impedance sensing signals may be

7   isolated from the driving signals. For example, a constant current at sensing frequency f_s

8   may be applied to integrated electrode pairs to be measured. The sensing electronics 88 ,

9   may then be operated at only the applied frequency f_s to determine a voltage drops across

10 the integrated electrode pairs, thus allowing the impedance at the sensing frequency f_s to

11 be derived without interference from the driving signals.

12 In another embodiment, driving signals may be used to monitor electrical

13 impedance directly. Driving signals may be switched to one or more integrated

14 electrodes to generate a force to manipulate or interact packets upon a reaction surface.

15 Simultaneously, an electrical current sensing circuit may be used to measure electrical

16 current going through the energized integrated electrodes. Electrode impedances may be

17 derived from such measurements of electrical current.

18 Although any suitable device may be used, in one embodiment a function

19 generator is used as signal generator **82**. More particularly, an arbitrary waveform signal

20 generator in combination with voltage or power amplifies or a transformer may be used to

21 generate the required voltages. In one embodiment, signal generator **82** may provide

22 sine-wave signals having a frequency up to the range of GHz and more particularly

23 between about 1 kHz and about 10 MHz and a voltage between about 1 V peak-to-peak

24 and about 1000 V peak-to-peak, and more particularly, between about 10 V peak-to-peak

25 and about 100 V peak-to-peak.

26 As illustrated, signal generator **82** may be connected to an electrode selector **84**.

27 Electrode selector **84** may apply one or more signals from signal generator **82** to one or

28 more individual electrodes (impedance sensing electrodes and/or driving electrodes may

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be individually addressable). Electrode selector **84** may be one of a number of suitable devices including a switch, a multiplexer, or the like. Alternatively, electrode selector **84** may apply one or more signals to one or more groups of electrodes. In one embodiment, selector **84** is made of electronic switches or a multiplexer. Selector **84** may be digitally controlled. With the benefit of this disclosure, those of skill in the art will understand that selector **84** may be any suitable device that may programmably divert one or more signals to one or more electrodes in any arbitrary manner.

As illustrated in **FIG. 6**, controller **81** provides a feedback loop mechanism from impedance sensing electrodes **19** to driving electrodes 18 via computer **80**, which itself is coupled to signal generator **82**, selector **84**, and transducer **88**. With the benefit of the present disclosure, those of skill in the art will recognize that controller **81** may contain more or fewer components. The feedback mechanism allows computer **80** to tailor its commands to signal generator **82** according to positions of packets upon reaction surface **12**, as determined by impedance sensing electrodes **19**. Thus, controller **81** allows for the adjustment of driving signals (and hence the adjustment of programmable manipulation forces) according to positions of packets (as determined by impedance sensing electrodes **19**). In embodiments utilizing an integral array of electrodes having integral impedance sensing electrodes 19 and driving electrodes 18, a feedback mechanism may operate as follows. Positions of packets may be determined by measuring impedances between electrical elements by applying impedance sensing signals to the integral array. Position information may then be used to control driving signals to the integral array to perform microfluidic processing through the manipulation of packets. In one embodiment computer **80** may be replaced by an application specific integrated circuit controller (ASIC) designed specifically for the purpose.

FIG. 7 shows an electrode driver **94** according to an embodiment of the presently disclosed method and apparatus. Driver **94** includes a computer **80**, a signal generator **82**, a resistor network **100**, a switching network **104**, and a bitmap **108**. Driver **94** is coupled to fluidic device **10** which includes reaction surface **12** and an integral array **43**.

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Driver **94** may assist in the application of signals to integral array **43** in order to direct microfluidic interactions of packets of material upon reaction surface **12**. In one embodiment, computer **80** directs signal generator **82** to apply an AC signal to integral array **43**. In the illustrated embodiment, from signal generator **82** there may be provided, for example, eight increasing voltage amplitudes using resistor network **100**, although more or fewer voltage amplitudes may be used . The eight AC signals may be distributed by switching network **104** *via* connection **106** to the integral array **43** according to a bitmap **108** or according to any other suitable data structure stored in computer **80** or in another device. By modifying bitmap **108** *via* computer **80**, different voltage amplitudes may be applied to different electrodes.

In one embodiment, signals to each electrode of integral array **43** may be represented in bitmap **108** by 3 bits to address eight available voltage amplitudes. Voltage amplitude distributions of bitmap **108** may be transmitted sequentially to switching network **104** *via* connection **110** twelve bits at a time using a communication protocol as is known in the art. In one embodiment, the communication protocol may use the following convention. To address a single electrode of integral array **43**, the first four bits may specify the row of the array. The second four bits may specify the column of the array. The next three bits may specify the desired voltage to be applied. The last bit may be used for error control by parity check. The rows/column arrangement may be used for different layouts of arrays. For instance, the row/column convention of addressing may be used even for a hexagonal grid array configuration. Those skilled in the art will appreciate that other methods may be used to address the electronic switching network **104** from computer **80**.

FIG. 8 is side cross-section view of one embodiment of a fluidic device **10**. Fluidic device **10** includes a wall **22** which encloses the sides and top of a reaction surface **12** to form a reaction chamber **41**. Reaction surface **12** includes an integral array **43**. Coupled to the integral array may be an interface board **54**. Interface board 54 may interface the integral array **43** with integrated circuits **50** *via* interconnect **55** and solder bumps **52**.

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In the embodiment of **FIG. 8**, interface board **54** may be sandwiched between chamber **41** and integrated circuits **50**. On one side, interface board **54** may provide electrical signals (AC or DC) to electrodes of integral array **43**, while the other side of interface board **54** may include pads for flip-chip mounting of integrated circuits **50**. Intermediate layers of interface board **54** may contain electrical leads, interconnects and vias, such as interconnect **55** to transfer power and signals to and from electrodes of integral array **43** and integrated circuits **50**.

Interface board **54** may be fabricated using suitable PC-board and flip chip technologies as is known in the art. Suitable silk-screened or electroplated flip-chip solder bump techniques may likewise be used. Alternatively, ink-jet solder deposition may be used as is known in the art.

FIG. 9 is a top view of an embodiment of a fluidic device **10**. In the illustrated embodiment, fluidic device **10** is made up of four distinct 8 by 8 integral arrays **43**, forming a 16 by 16 array. Under each 8 by 8 array may be situated an integrated circuit (not shown in **FIG. 9**) that may provide control and signal processing to electrodes of the integral array **43**. The integral arrays may be coupled to a circuit conducting pad **34** that may be coupled to an interface conducting pad **36** by a bond wire **38** (shown only in one quadrant). Connected to interface conducting pad **36** may be wire **42**, or another suitable connector such as a PC board connector, leading to a computer or other suitable controlling device.

FIG. 9B is another top view of an embodiment of a fluidic device **10**. In this embodiment, many ports **15** are situated along edges of fluidic device **10**. These ports **15** may serve to inject and/or collect packets **21** to/from reaction surface **12**. Also illustrated is a sensor **122** positioned adjacent a port **15**. Such a sensor is described in reference to **FIG. 10** below.

FIG. 10 is a block diagram of a microfluidic processing system **115**. Processing system **115** may be designed to allow for control of programmable dielectrophoretic array (PDA) **116** that serves as the site for microfluidic interactions and may be constructed in

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1 accordance with the present disclosure. In view of its broad functionality, PDA **116** may

2 serve a role, in the field of fluidic processing, analogous to the role played by a Central

3 Processing Unit in the field of computers.

4 Coupled to PDA **116** are fluidic sensors **122**. Fluidic sensors **122** may measure

5 and monitor fluid products from, in, or on PDA **116**. For instance, fluidic sensors **122**

6 may measure and identify reaction products and may quantify reactions between packets.

7 In one embodiment, fluidic sensors **122** may include an optical microscope or one or

8 more sensors (chemical, electrochemical, electrical, optical, or the like), but any other

9 suitable monitoring device known in the art may be substituted therewith. For example,

10 fluidic sensors **122** may be an electrochemical sensor that monitors the presence and

11 concentration of electroactive (redox-active) molecules in a packet solution. An

12 electrochemical sensor may take the form of two or more microelectrodes. In a three-

13 electrode configuration, for example, electrodes may correspond to working, reference,

14 and counter electrodes. A packet to be analyzed may be moved to be in contact with the

15 three electrodes. A voltage signal may be applied between the working and reference

16 electrode, and the current between the working and counter electrode may be monitored.

17 The voltage-current relationship allows for the determination of the presence or absence,

18 and concentration of electro-active molecules in the packet solution. Also attached to

19 PDA **116** may be suitable material injection and extraction devices **120** coupled to

20 appropriate inlet or outlet ports of PDA **116** (not shown in **FIG. 10**). Such devices may

21 be any suitable structure allowing ingress to and egress from PDA **116**.

22 In electrical communication with PDA **116** may be PDA voltage drivers **126** and

23 dielectric position sensors **124**. PDA voltage drivers **126** may be adapted to drive

24 electrodes within PDA **116** so that an electric field may be established that sets up

25 manipulation forces that manipulate one or more packets of material within PDA **116** to

26 promote microfluidic interactions. In one embodiment, PDA voltage drivers **126** may

27 include a signal generator and switching network as described in relation to **FIG. 7**.

28 Dielectric position sensors **124** may measure positions of packets within PDA **116**. In

29 one embodiment, dielectric position sensors **124** may include measuring devices

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1   connected to appropriate sensors that may determine a position of one or more packets of

2   material by sensing, for instance, a change in impedance between neighboring impedance

3   sensing electrodes within PDA **116** and by correlating that change in impedance with a

4   packet positioned adjacent the neighboring sensors according to the teachings of the

5   present disclosure.

6   Coupled to packet injection and extraction devices **120**, PDA voltage drivers **126**,

7   and dielectric position sensors **124** may be computer interface **128**. Computer

8   interface **128** may be configured to allow host computer **130** to interact with PDA **116**.

9   In one embodiment, computer interface **128** may be a digital or analog card or board that

10   may analyze impedance data to obtain a packet distribution map.

11   In the embodiment of **FIG. 10**, host computer **130** may be coupled to computer

12   interface **128** to provide for control of PDA **116**. Host computer **130** may be coupled to

13   position tracking agent **132** and to low-level control agent **134**. Position tracking agent

14   **132** may be adapted to store, process, and track positions of packets within the fluidic

15   processor PDA **116**. Low-level control agent **134** may be configured to provide

16   instructions to host computer **130** from library interface **136** and software interface **138**.

17   Library interface **136** may hold various sets of subroutines for programmably

18   manipulating packets of materials on PDA **116**. Software interface **138** that may allow

19   for custom programming of instructions to be executed by the fluidic processor PDA **116**

20   to programmably manipulate packets. Alternatively established programs of

21   manipulation instructions for specific fluid processing tests may be read from stored data

22   and executed by the PDA fluid processor **116**.

23   **FIG. 11** illustrates operation of the presently disclosed method and apparatus. In

24   **FIG. 11**, open squares represent electrodes of an integral array. However, it is

25   contemplated that the description below applies equally well to a device utilizing separate

26   impedance sensing electrodes and driving electrodes.

27   In the illustrated embodiment, a packet **21a** may be introduced onto reaction

28   surface **12** adjacent the location represented by integral impedance sensor/electrode **201**.

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1   The packet may be compartmentalized in an immiscible partitioning medium (not

2   shown). The introduction of the packet may be accomplished using an appropriate inlet

3   port positioned adjacent to electrode **201**. Alternatively, a packet may be introduced

4   adjacent electrode **201** by applying an appropriate signal to electrode **201** to generate an

5   extraction force that may extract the packet from an inlet port or from an injector directly

6   onto reaction surface **12** and adjacent to electrode **201**.

7   Once positioned upon reaction surface **12**, packet **21a** may be made to move along

8   a predetermined path indicated by dashed line **250**. A path may be specified in a number

9   of different ways. In one embodiment, a user may specifically define a path. For

10 instance, one may specify a path, through appropriate programming of a controller or

11 processing system, such as the one depicted numeral **250**. Alternatively, a user may

12 specify a starting position and an ending position to define a path. For instance, a user

13 may specify that packet **21a** is to be introduced adjacent electrode **201** and end at a

14 location adjacent electrode **215**. Alternatively, one may specify a starting and ending

15 location with specific path information in between. For instance, a user may specify a

16 starting position, an ending position, and a wavy path in between. As can be seen from

17 **FIG. 11**, the path may have any arbitrary shape and it may be programmed in any number

18 of ways.

19 To move packet **21a** generally along the path, electrical signals may be suitably

20 switched to integral impedance sensors/electrode pairs so that programmable

21 manipulation forces may be created that act upon packet **21a** to propel it generally along

22 the specified path. As discussed earlier, the signals may be varied in numerous ways to

23 achieve the proper manipulation force. In the illustrated embodiment, applying voltage

24 signals to electrode pairs **202** and **203** may create an attractive dielectrophoretic force that

25 moves packet **21a** from electrode **201** towards electrode **203** generally along path **250**.

26 As packet **21a** moves generally along a specified path, the integral array may measure

27 impedances to map the position of the packet upon reaction surface **12**. Knowing the

28 position of a packet allows manipulation forces to be directed at appropriate positions to

29 achieve a desired microfluidic processing task or interaction. In particular, knowing a

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1 position of a packet allows an appropriate signal to be switched to an appropriate

2 electrode or electrode pair to generate a manipulation force that further propels or

3 interacts the packet according to one or more instructions.

4 As packet **21a** moves from electrode **201** towards electrode **203**, the impedance

5 between electrode **202** and electrode **203** may change value, indicating that packet **21a** is

6 between, or partially between, those two electrodes. The impedance may be measured as

7 described in **FIG. 3**. A controller or processing system (not shown in **FIG. 11**) may

8 register the location of packet **21a** and may apply a signal, for instance, to electrode pairs

9 **204** and **205**, creating an attractive dielectrophoretic force which propels packet **21a**

10 towards those electrodes generally along path **250**. As the impedance between electrode

11 **204** and electrode **205** changes value, a controller or processing system may apply a

12 signal to electrodes **206** and **207** to propel packet **21a** along path **250**. As packet **21a**

13 continues along path **250**, the impedance between electrode **206** and electrode **207** may

14 change value, indicating the presence of packet **21a** adjacent that location along the array.

15 Thus, as packet **21a** moves along path **250**, a controller or processing system may

16 constantly monitor the position of the packet by measuring impedance between electrode

17 pairs and adjust electrical signals to an appropriate electrode or electrode pair (and hence,

18 adjust manipulation forces) to continue to propel the packet further along the specified

19 path.

20 Measuring an impedance between pairs of electrodes not only allows a position of

21 a packet to be determined, but it also allows for the determination of a location of an

22 obstruction or blockage upon reaction surface **12**. For example, measuring the impedance

23 between electrodes **211** and **213** may indicate the presence of obstruction **212**. By noting

24 the position of obstruction **212**, a controller or processing system may re-route one or

25 more packets around the obstruction so that no interference with microfluidic processing

26 interactions occurs. For example, if a path is specified that passes through an area

27 occupied by obstruction **212**, a controller or processing system may modify electrical

28 signals to propel a packet generally along the specified path while avoiding the

29 obstruction. For instance, a stronger or weaker signal may be sent to one or more

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1 electrodes or electrode pairs near obstruction **212** to steer a packet clear of the blockage
2 while still maintaining, generally, the path that was originally specified, and more
3 particularly, the originally specified end point

4 A controller or processing system according to the presently disclosed method and
5 apparatus may be programmed to scan for several obstructions and/or packets. Such a
6 scan may build up a distribution map, showing the location(s) of various packets and/or
7 obstructions on an entire reaction surface **12** or a portion thereof. Such a distribution map
8 may be a virtual map, stored, for example, in a computer memory or display. Turning
9 again to **FIG. 11**, impedances of all electrode pairs adjacent to path **250** may be measured
10 to determine if an obstruction blocks that path or if a packet lies somewhere in that area.
11 If the path is determined to be clear (e.g., if all the electrode pairs show an impedance
12 value indicating a clear area), a packet may be safely propelled generally along the path
13 while avoiding any interactions with other packets and/or obstructions. However, if an
14 obstruction is discovered, several different actions may be taken. In one embodiment, the
15 user may be notified that a blockage exists along the specified path. The user may then
16 specify a different path or give another appropriate instruction. In another embodiment,
17 the controller or processing system may determine if the obstruction may be avoided
18 while still maintaining generally the same specified path. If possible, electrical signals
19 may be modified and delivered to an electrode or electrode pairs to generate appropriate
20 electrical field distributions that set up proper manipulation forces that will aid in
21 avoiding the obstruction. Because, at least in part, of this ability to constantly measure
22 positions and responses of packets during manipulation, a controller or processing system
23 may be capable of monitoring the integrity of fluidic processing, reporting and correcting
24 any errors that may occur.

25 **FIG. 11** also depicts how processing may be carried out on two packets. In the
26 illustrated embodiment, a second packet **21b** begins on reaction surface **12** near electrode
27 **217**. A second path, path **260**, may be specified that ends at electrode **219**. As can be
28 seen, paths **250** and **260** may cross at interaction point **240**. At interaction point **240**, the
29 two packets may interact in many ways as illustrated, for example, in **FIG. 12**. The

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1   interaction may include, but is not limited to, fusing, merging, mixing, reacting, dividing,

2   splitting, or any combination thereof. For instance, the two packets may interact at

3   interaction point **240** to form one or more intermediate or final reaction products. Those

4   products may be manipulated in the same or in a similar manner as the two original

5   packets were manipulated.

6   **FIG. 11** also depicts how maintenance may be performed upon reaction

7   surface **12**. A maintenance packet **21c** adapted to perform maintenance upon reaction

8   surface **12** may be introduced onto reaction surface **12** by a maintenance port (not shown

9   in **FIG. 11**). A maintenance port may be similar to an inlet port in structure but may be

10   dedicated to the introduction of one or more maintenance packets **21c** designed

11   specifically, for instance, to clean or maintain reaction surface **12**, a surface coating, or

12   one or more electrodes or sensors. Maintenance packet **21c** may also react with an

13   obstruction in such a way as to remove that obstruction. As illustrated, maintenance

14   packet **21c** may begin near electrode **241**. It may then be propelled along path **270**,

15   providing maintenance, perhaps, to electrodes **242** and **243**. Maintenance packet **21c** may

16   be propelled back to a maintenance port, extracted from reaction surface **12**, and later

17   used again, or it may discarded at an outlet part.

18   **FIG. 12** demonstrates several different possible fluidic interactions that may be

19   carried out using the presently disclosed method and apparatus. In the illustrated

20   embodiment, packets **21** (only one is labeled for convenience) reside upon a reaction

21   surface **12** having an integral array **43** (only one electrode is labeled for convenience). In

22   the top pane of **FIG. 12**, there is shown an interaction in which a single packet is

23   manipulated on the reaction surface by moving the packet in a programmed fashion. In

24   the middle pane, two packets, starting at different locations upon the reaction surface, are

25   directed, via appropriate electrical signals, to come together at a specified location (near

26   the center of the array) to fuse together, for example, to initiate a reaction. The fused

27   packet may be manipulated just as the original packets were manipulated. For instance,

28   the fused packet may be moved to various locations or it may fuse again with another

29   packet(s). Shown in the bottom pane of **FIG. 12** is a splitting interaction. As shown, a

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1   single packet is subjected to different programmable manipulation forces that cause the

2   packet to split into two distinct packets. Such an interaction may be accomplished by,

3   first, noting the position of the packet to be split, and then by applying appropriate signals

4   to electrode pairs to generate two or more opposing forces that cause the packet to split

5   apart.

6   **FIG. 13** is a flowchart showing one embodiment of a method of operation. A

7   material may be introduced onto a reaction surface and compartmentalized to form one or

8   more packets in step **300**. Multiple materials may be introduced at different locations

9   along reaction surface **12** to form a plurality of packets. A path may be specified as in

10   step **310**. The path may be designed to accomplish any type of microfluidic processing,

11   manipulation, or interaction. Different reactions may be performed in serial or in parallel

12   according to different paths. Instructions governing such processing may be embodied in

13   the pseudo-code that may be routed through computer interface **128** of **FIG. 10**.

14   Illustrative code may read as follows:

15   Example: *AvidinActin.PSL*

```
16   Use inlet(1-3), outlet(1-2)
17   Inlet(1) is actin
18   Inlet(2) is avidin
19   Inlet(3) is enzyme
20   Outlet(1) is polymer
21   Outlet(2) is waste
22   Matrix(1,2) is accumulator
23   Clean
24   Do
25       Sactin = (Pull actin)                              // pull a new packet on the next
26       Savidin = (Pull avidin)                            // available matrix element next to
27       Senzyme = (Pull enzyme)                            // the inlets
28       Move Sactin into accumulator                       // merges components and enzyme
29       Move Savidin into accumulator                      // in a single packet
30       Move Senzyme into accumulator
31       Wait 1000ms
32       ShiftRow accumulator.row ,+1                       // drag packet left into polymer outlet
33       Move 0.5*accumulator into (2, accumulator.column)// drag half packet to row 2
34       ShiftRow 2, + 1                                    // drag packet left into waste
35   Loop Until polymer.count = 10                          // number of packet at polymer outlet = 10
36   Clean
```

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1   In step **315**, computer **80** of **FIG. 6** or any other suitable device may determine

2   the next unit step along the path specified in step **315**. In other words, a path may be

3   broken down into unit steps and the next unit step or steps may be determined with

4   respect to the specified path. In step **320**, a programmable manipulation force is

5   generated on reaction surface **12** through the use of any of the mechanisms disclosed

6   herein. The programmable manipulation force may manipulate the one or more packets

7   according to instructions from a user. In step **330**, the response(s) of the one or more

8   packets may be monitored. This step may include measuring an impedance on the

9   reaction surface as discussed herein. In particular, one may determine whether the one or

10   more packets moved to where they were supposed to, or whether they interacted as

11   instructed. In step **340**, it may be determined if the packet movement was successful --

12   that is, it may be determined whether the packet ended up at a location corresponding to

13   the unit step determined in step **315**.

14   If a packet movement was successful (i.e., if the packet responded correctly to the

15   programmable manipulation force(s)), then it may be determined, by comparison with the

16   specified path, whether or not the packet destination has been reached. In other words, it

17   may be determined if the packet has moved to the end location of the specified path. If

18   the destination has not been reached, the next unit step movement may be determined at

19   step **315** and steps **320**, **330**, **340**, and **365** may be repeated. If the destination has been

20   reached, it may be determined whether another packet is to be manipulated in step **370**.

21   This step may include a user prompt. If no further packets are to be manipulated, it may

22   be determined whether fluidic processing is complete in step **380**. If yes, the process may

23   be ended at step **390**. Step **390** may include the collecting of one or more packets, further

24   analysis, throwing away of the reaction surface, or any procedure described herein. If the

25   processing is not complete, the next step of processing may be determined in step **395**.

26   The next step may entail, for example, the introduction of another packet, the

27   specification of another path, or any other step of **FIG. 13**.

28   If a packet manipulation is unsuccessful (i.e., if the applied programmable

29   manipulation force(s) did not produce a desired interaction or movement along a

specified path as indicated by step **340**), one may locate an obstruction upon the reaction surface as indicated in step **350** and as taught herein. After locating any obstructions, a new, modified path may be determined or specified as indicated by step **360**, leading to step **310**.

As mentioned with relation to **FIG. 1**, the present disclosure contemplates that many different types of forces may be utilized as a manipulation force for promoting fluidic interactions among packets of material on a reaction surface. Specifically, suitable forces other than dielectrophoresis include electrophoretic forces, optical forces, mechanical forces, or any combination thereof. Below are discussed embodiments of the present disclosure dealing with electrophoretic and optical manipulation forces.

Programmable Electrophoretic Array (PEA)

A fluidic processing system incorporating a programmable electrophoretic array may be constructed according to the present disclosure. As used herein, "programmable electrophoretic array" (PEA) refers to an electrode array whose individual elements can be addressed with DC, pulsed, or low frequency AC electrical signals (typically, less than about 10 kHz) electrical signals. The addressing of electrode elements with electrical signals initiates different field distributions and generates electrophoretic manipulation forces that trap, repel, transport or perform other manipulations upon charged packets on and above the electrode plane. By programmably addressing electrode elements within the array with electrical signals, electric field distributions and electrophoretic manipulation forces acting upon charged packets may be programmable so that packets may be manipulated along arbitrarily chosen or predetermined paths. A PEA may utilize electrophoretic forces in DC or low-frequency (typically, less than about 10 kHz) AC electrical fields. Such electrophoretic forces may be used instead of, or in addition to, another manipulation forces such as dielectrophoresis.

Negative or positive charges may be induced or injected into fluid packets. The charged packets may be moved or manipulated by electrophoretic forces generated by an electrode array fabricated on an inner surfaces of a chamber in accordance with this

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1   disclosure. The electrode array, termed a programmable electrophoretic array (PEA),

2   may consist of uniformly or non-uniformly spaced electrode elements. Individual

3   electrode elements may be independently addressable with DC, pulsed, or low frequency

4   AC electrical signals (< about 10 kHz). Characteristic dimensions of individual electrode

5   elements may be of any size but, in one embodiment, may lie between 0.2 micron and 10

6   mm. Individual electrode elements may take similar or different geometrical forms such

7   as squares, circles, diamonds, or other shapes. Programmably switchable electrical

8   signals may be applied to individual electrode elements so that a programmable electrical

9   field distribution may be generated. Such a distribution may impose electrophoretic

10   forces to trap, repel, transport or manipulate charged packets in a partitioning medium.

11   Further, electrical signals may be applied to such an array so that a packet may be broken

12   down to two or more packets. The programmability of a PEA may be reflected in the fact

13   that the electric field distributions and electrophoretic forces acting on charged packets

14   may be programmable so that charged packets may be trapped or repelled or transported

15   along arbitrarily chosen paths in the partitioning medium, and that a PEA may be

16   programmed to perform different reactions in series or in parallel where different

17   manipulation protocols of packets (differing in size, number, and/or reagent type

18   concentration) may be required. As with PDA surface modification, if a dielectric layer

19   coating is applied to the surface of a PEA to modify interaction forces between packets

20   reaction surfaces, the dielectric layer may be made sufficiently thin (typically 2 nm to 1

21   micron) to allow for electric field penetration.

22   *Optical Manipulation*

23   Optical tweezers (which may consist of a focused laser beam with a light intensity

24   gradient) may be also be used for trapping and manipulating packets of material. Optical

25   manipulation requires that the refractive indices of the packets be different from that of

26   their suspending medium, for instance, a partitioning medium as described herein. As

27   light passes through one or more packets, it may induce fluctuating dipoles. Those

28   dipoles may interact with electromagnetic field gradients, resulting in optical forces

29   directed towards or away from the brighter region of the light. If their refractive indices

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1 are higher than that of the partitioning medium, packets may be trapped in a bright

2 region, and when the laser light moves with respect to the partitioning medium, packets

3 may follow the light beam, allowing for optical manipulation forces. Conversely, if the

4 packets have refractive indices smaller than their partitioning medium, they will

5 experience forces directing them away from bright regions.

6 Therefore, if packets have different refractive indexes from that of the partitioning

7 medium (e.g., water packets in air or oil), optical tweezers may exert forces on them.

8 Therefore, to manipulate and interact packets, a microscope or other optical system

9 incorporating one or more laser tweezers may be used. A chamber containing a

10 partitioning medium in accordance with the present disclosure may be placed into such an

11 optical system. Following the introduction of packets of material into the chamber, laser

12 tweezers may be used to trap packets. By moving the focal point of the optical tweezers

13 with respect to the partitioning medium (e.g., moving a stage holding the thin chamber

14 containing the partitioning medium whilst fixing the position of laser tweezers and/or by

15 focusing the laser beam to different depths in the partitioning medium), packets may be

16 manipulated as described herein. Through the use of apparatus such as a computer-

17 controllable, multi-axis translation stage, the movement of the optical tweezers with

18 respect to the suspending medium may be programmed or automatically controlled. Thus

19 the optical tweezer may be moved, with respect to the medium, along any arbitrarily

20 chosen or predetermined paths. By doing so, packets under the influences of the optical

21 tweezers may be manipulated along any arbitrarily chosen or predetermined paths.

22 **Example 1**

23 Aqueous materials have been compartmentalized to form packets using

24 hydrophobic liquids as a partitioning medium. Partitioning mediums so used have

25 included decane, bromodocane, mineral oil, and 3 in 1™ oil. Packets have been formed

26 by briefly sonicating about 3 milliliters of the hydrophobic liquid to which had been

27 added 20 to 50 microliters of aqueous medium. Aqueous media tested have included

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1 deionized water, tap water (electrical conductivity of about 40 mS/m) and phosphate

2 buffered saline (PBS) solution.

3 **Example 2**

4 Aqueous packets suspended in mineral oil, bromodoecane and 3 in 1™ oil have

5 been collected by dielectrophoresis by applying sinusoidal signals to gold-on-glass

6 electrode arrays having 20, 80 and 160 micron spacing, respectively. The 20-micron

7 electrode array consisted of parallel line electrodes (20 microns in width and spacing).

8 The 80 and 160 micron electrode arrays were of the interdigitated, castellated geometries.

9 Aqueous packets were collected at electrode edges or tips when AC voltage signals

10 between 100 Hz and 20 MHz were applied. Applied voltages were from 10 to 100 V

11 peak-to-peak. The formation of pearl-chains of water packets has also been observed.

12 **Example 3**

13 Aqueous packets in hydrophobic suspension have been brought together and fused

14 under the influence of dielectrophoretic forces on the same electrode arrays used in

15 Example 2.

16 **Example 4**

17 Packets have been moved from one electrode element to another under influence

18 of dielectrophoretic forces when the AC electrical field is switched on an addressable

19 array of parallel line electrodes having 20 micron width and spacing.

20 **Example 5**

21 Sensitive AC impedance monitors have been built for use with microelectrode

22 arrays. Such monitors may provide for sensitive dielectric sensing of packet positions.

23 While the present disclosure may be adaptable to various modifications and

24 alternative forms, specific embodiments have been shown by way of example and

25 described herein. However, it should be understood that the present disclosure is not

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1   intended to be limited to the particular forms disclosed. Rather, it is to cover all

2   modifications, equivalents, and alternatives falling within the spirit and scope of the

3   disclosure as defined by the appended claims. Moreover, the different aspects of the

4   disclosed apparatus and methods may be utilized in various combinations and/or

5   independently. Thus the invention is not limited to only those combinations shown

6   herein, but rather may include other combinations.